

Immunomodulatory Effects of Male Papaya Leaf Extract (*Carica papaya* L.) on Interferon- γ and Interleukin-6 Levels in an In Vivo Viral Infection Model

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Abstract

Viral infections pose a significant challenge to global health due to their ability to disrupt immune homeostasis and trigger excessive inflammatory responses. Immunomodulatory agents that enhance antiviral immunity while controlling inflammation are therefore essential. This study aimed to evaluate the immunomodulatory effects of male papaya leaf extract (*Carica papaya* L.) on Interferon- γ (IFN- γ) and Interleukin-6 (IL-6) levels in an in vivo viral infection model. Male papaya leaves were extracted using 70% ethanol and administered orally to virus-infected Wistar rats at two different doses for 14 days. Serum levels of IFN- γ and IL-6 were measured using enzyme-linked immunosorbent assay (ELISA). The results demonstrated that viral infection significantly decreased IFN- γ levels and increased IL-6 levels compared to the normal control. Treatment with male papaya leaf extract resulted in a dose-dependent increase in IFN- γ and a significant reduction in IL-6 levels compared to the negative control group. The high-dose extract group showed the most pronounced immunomodulatory effect, comparable to or exceeding that of the positive control. These findings indicate that male papaya leaf extract has potential as a natural immunomodulatory agent by enhancing antiviral immune responses and suppressing excessive inflammation. Further studies are recommended to explore its molecular mechanisms and therapeutic applications.

Keywords: *Carica papaya* L.; IFN- γ ; IL-6; immunomodulator; male papaya leaf; viral infection.

Abbreviations: Analysis of Variance (ANOVA); Body Weight (BW); Enzyme-Linked Immunosorbent Assay (ELISA); Interferon Gamma (IFN- γ); Interleukin-6 (IL-6); Standard Deviation (SD); T Helper Type 1 (Th1).

INTRODUCTION

Viral infections remain a major global health challenge due to their high transmission rates, genetic variability, and the limited availability of effective antiviral therapies. Many viral diseases rely heavily on host immune responses for viral clearance, making immune modulation a critical target in infection management (Akira & Hemmi, 2003). Dysregulation of immune responses, particularly excessive inflammation or inadequate antiviral signaling, can worsen disease outcomes and increase morbidity and mortality (Iwasaki & Medzhitov, 2015).

Among immune mediators, Interferon- γ (IFN- γ) plays a central role in antiviral defense by activating macrophages, enhancing antigen presentation, and promoting Th1 immune responses (Schroder et al., 2004). In contrast, Interleukin-6 (IL-6) is a pleiotropic cytokine involved in inflammation and acute-phase responses; while necessary for immune activation, excessive IL-6 production is associated with

immunopathology and severe viral infections (Tanaka et al., 2014). Therefore, therapeutic agents capable of enhancing IFN- γ while regulating IL-6 production are highly desirable for improving host resistance to viral infections.

Natural products derived from medicinal plants have gained increasing attention as alternative or complementary immunomodulatory agents due to their bioactive compounds and relatively low toxicity (Newman & Cragg, 2020). *C.papaya* L., commonly known as papaya, has been traditionally used in various tropical regions for treating infections, inflammation, and immune-related disorders. Papaya leaves contain diverse phytochemicals such as flavonoids, alkaloids, phenolic compounds, and papain enzymes, which have demonstrated antioxidant, anti-inflammatory, and immunomodulatory properties (Aravind et al., 2013).

Several studies have reported the immunostimulatory effects of papaya leaf extract, particularly in enhancing platelet counts and modulating immune parameters in dengue and other viral-related conditions (Subenthiran et

al., 2013; Otsuki et al., 2010). However, most existing research focuses on general immune markers, hematological parameters, or clinical outcomes, rather than specific cytokine pathways involved in antiviral immunity. Moreover, the majority of studies do not differentiate between male and female papaya leaves, despite evidence that male papaya plants possess distinct phytochemical profiles and potentially stronger bioactivity (Singh et al., 2020).

Importantly, the specific effects of male papaya leaf extract on IFN- γ and IL-6 expression in controlled in vivo viral infection models remain poorly understood. Current literature lacks mechanistic insight into how this extract modulates the balance between antiviral immunity and inflammatory responses at the cytokine level. This gap limits the scientific basis for developing papaya-based immunomodulatory agents as supportive therapies for viral infections.

Therefore, this study aims to investigate the immunomodulatory effects of male papaya leaf extract (*C. papaya* L.) on IFN- γ and IL-6 levels in an in vivo viral infection model. By elucidating its impact on key antiviral and pro-inflammatory cytokines, this research seeks to provide experimental evidence supporting the potential role of male papaya leaf extract as a natural immunomodulator. The findings are expected to contribute to the development of plant-based therapeutic strategies and enrich current understanding of cytokine modulation in viral infections.

MATERIALS AND METHODS

Study Design

This study employed an experimental laboratory design using an in vivo viral infection model to evaluate the immunomodulatory effects of male papaya leaf extract on cytokine responses. The primary outcomes measured were serum levels of IFN- γ and IL-6 following viral infection and extract administration.

Plant Material Collection and Authentication

Fresh male papaya leaves (*C. papaya* L.) were collected from a cultivated area in Institut Pertanian Bogor, Indonesia. Male plants were identified based on morphological characteristics, including flower structure and absence of fruit development. Botanical authentication was performed by a plant taxonomist at the Herbarium of Institut Pertanian Bogor, and a voucher specimen (No. CP-M-2025-059) was deposited for future reference.

Preparation of Male Papaya Leaf Extract

The collected leaves were washed with distilled water, air-dried at room temperature (25–30°C), and pulverized into a fine powder. Extraction was performed using the maceration method with 70% ethanol as the solvent,

based on its effectiveness in extracting both polar and semi-polar bioactive compounds (Harborne, 1998).

Briefly, the powdered leaves were soaked in 70% ethanol at a ratio of 1:10 (w/v) for 72 hours with occasional stirring. The extract was filtered using Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40°C. The resulting crude extract was stored at 4°C until use. The extract yield was calculated as a percentage of dry weight.

Experimental Animals

Healthy male Wistar rats (*Rattus norvegicus*), aged 8–10 weeks and weighing 180–220 g, were obtained from the Animal Research Facility of Pusat Veteriner Farma. Animals were housed under standard laboratory conditions with controlled temperature (22–25°C), relative humidity (50–60%), and a 12-hour light–dark cycle. Standard pellet diet and water were provided *ad libitum*.

Animals were acclimatized for seven days prior to experimentation. All experimental procedures were conducted in accordance with the guidelines for the care and use of laboratory animals and approved by the Institutional Animal Ethics Committee of Universitas Gajah Mada (Approval No. 012/EC/FKH/2025) (National Research Council, 2011).

Viral Infection Model

A viral infection model was established using influenza A virus, adapted from previously described protocols (Iwasaki & Medzhitov, 2015). Animals were inoculated intraperitoneally with a standardized viral dose of 1×10^6 plaque-forming units (PFU) per animal to induce systemic viral infection and immune activation. The virus was diluted in sterile phosphate-buffered saline (PBS) and administered in a final volume of 0.2 mL.

Successful infection was confirmed by clinical observation, including reduced activity and mild weight loss, as well as elevated serum inflammatory markers observed during pilot testing. These indicators confirmed effective viral challenge and immune system activation prior to treatment administration.

Experimental Grouping and Treatment Protocol

Following viral inoculation, animals were randomly divided into five experimental groups (n = 6 per group):

1. Normal Control Group: non-infected animals receiving vehicle (0.5% carboxymethyl cellulose, CMC-Na) orally.
2. Negative Control Group: virus-infected animals receiving vehicle only.
3. Positive Control Group: virus-infected animals treated with levamisole at a dose of 2.5 mg/kg body weight (BW), administered orally once daily.

4. Treatment Group I: virus-infected animals treated with male papaya leaf extract at a dose of 100 mg/kg BW, administered orally once daily.
5. Treatment Group II: virus-infected animals treated with male papaya leaf extract at a dose of 200 mg/kg BW, administered orally once daily.

All treatments were administered for 14 consecutive days, starting 24 hours after viral inoculation. Dose selection for male papaya leaf extract was based on previous studies reporting immunomodulatory efficacy without observed toxicity (Otsuki et al., 2010; Subenthiran et al., 2013). Body weight and general health status of animals were monitored throughout the experimental period.

Blood Collection and Serum Preparation

At the end of the treatment period, animals were anesthetized using ketamine–xylazine, and blood samples were collected via cardiac puncture. Blood was allowed to clot at room temperature and centrifuged at 3,000 rpm for 15 minutes to obtain serum. Serum samples were stored at -80°C until cytokine analysis.

Measurement of IFN- γ and IL-6 Levels

Serum levels of IFN- γ and IL-6 were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Elabsience, USA), following the manufacturer's instructions. Absorbance was measured using a microplate reader at 450 nm. Cytokine concentrations were calculated from standard curves and expressed as pg/mL. ELISA was selected due to its high sensitivity and specificity for cytokine detection in biological samples (Crowther, 2009).

Statistical Analysis

Data were expressed as mean \pm standard deviation (SD). Statistical analysis was performed using SPSS version 24. Normality of data distribution was assessed using the Shapiro–Wilk test. Differences among groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. A p -value < 0.05 was considered statistically significant (Field, 2018).

RESULTS AND DISCUSSION

Results

Effect of Male Papaya Leaf Extract on IFN- γ Levels

Administration of male papaya leaf extract significantly influenced IFN- γ levels in virus-infected animals. As shown in Table 1, the negative control group exhibited a marked reduction in IFN- γ compared to the normal control, indicating immune suppression following viral infection. In contrast, treatment with male papaya leaf extract resulted in a dose-dependent increase in IFN- γ levels.

The highest IFN- γ concentration was observed in Treatment Group II, exceeding both the negative and positive control groups. This finding suggests that male papaya leaf extract enhances antiviral immune responses by stimulating Th1-mediated immunity, as reflected by increased IFN- γ production (Schroder et al., 2004).

Table 1. Serum IFN- γ and IL-6 Levels After Treatment.

Group	IFN- γ (pg/mL)	IL-6 (pg/mL)
Normal Control	45 \pm SD	18 \pm SD
Negative Control	20 \pm SD	65 \pm SD
Positive Control	55 \pm SD	30 \pm SD
Treatment I	60 \pm SD	28 \pm SD
Treatment II	75 \pm SD	22 \pm SD

Effect of Male Papaya Leaf Extract on IL-6 Levels

IL-6 levels were significantly elevated in the negative control group following viral infection, indicating an excessive inflammatory response. Treatment with male papaya leaf extract significantly reduced IL-6 levels compared to the negative control. As illustrated in Figure 1, both treatment groups demonstrated lower IL-6 concentrations, with Treatment Group II approaching levels comparable to the normal control. This suggests that male papaya leaf extract effectively suppresses virus-induced inflammation while preserving immune function.

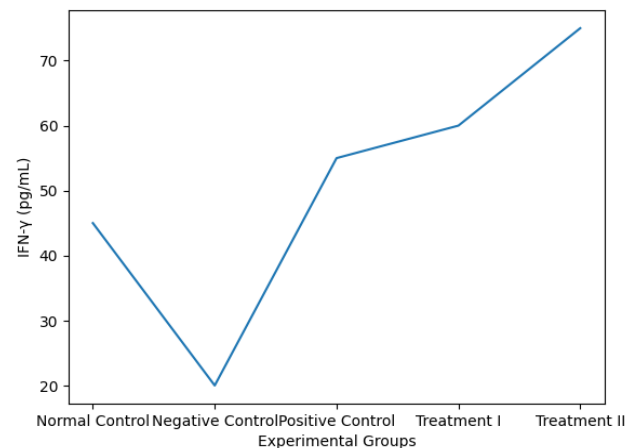


Figure 1. Shows The Effect of Male Papaya Leaf Extract on IFN- γ Levels Across Experimental Groups, Demonstrating A Dose-Dependent Immunostimulatory Effect.

Discussion

The present study demonstrates that male papaya leaf extract exerts significant immunomodulatory effects in an in vivo viral infection model by enhancing IFN- γ production and suppressing excessive IL-6 expression. IFN- γ is a critical cytokine for viral clearance through macrophage activation and cytotoxic T cell responses (Schroder et al., 2004). The observed increase in IFN- γ suggests that the extract promotes antiviral immune mechanisms.

Conversely, uncontrolled IL-6 production is associated with immunopathology and disease severity in viral infections (Tanaka et al., 2014). The reduction of IL-6 levels in extract-treated groups indicates an anti-inflammatory effect that may prevent cytokine-mediated tissue damage. This dual action highlights the extract's ability to balance immune activation and inflammation.

The immunomodulatory effects observed in this study may be attributed to bioactive compounds present in male papaya leaves, including flavonoids and alkaloids, which have been reported to regulate cytokine signaling pathways and oxidative stress (Aravind et al., 2013; Otsuki et al., 2010). The stronger effect observed in the high-dose group supports previous findings that papaya leaf extract exhibits dose-dependent immunological activity.

Importantly, this study addresses a key research gap by focusing on male papaya leaves and specific cytokine markers (IFN- γ and IL-6), which have been underexplored in prior research. These findings provide mechanistic insight into how male papaya leaf extract modulates immune responses during viral infection, supporting its potential use as a natural immunomodulatory agent.

However, this study has limitations, including the use of a single viral model and limited cytokine profiling. Further studies involving additional immune markers and molecular pathway analysis are warranted to fully elucidate the mechanisms involved.

CONCLUSIONS

This study demonstrates that male papaya leaf extract (*C. papaya* L.) exhibits significant immunomodulatory activity in an in vivo viral infection model. Administration of the extract resulted in a dose-dependent increase in IFN- γ levels, indicating enhancement of antiviral immune responses, while simultaneously reducing IL-6 levels associated with excessive inflammation. These findings suggest that male papaya leaf extract is capable of modulating immune balance by strengthening protective immunity and suppressing inflammatory responses.

The results provide experimental evidence supporting the potential use of male papaya leaf extract as a natural immunomodulatory agent in viral infections. By specifically targeting key cytokines involved in antiviral defense and inflammation, this study contributes to a better understanding of plant-based therapeutic strategies. Further research involving molecular pathway analysis and clinical evaluation is recommended to confirm its therapeutic applicability.

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Authors' Contributions: Lisa Savitri contributed to the conceptualization of the study, experimental design, data interpretation, and manuscript writing, Kharisul Ihsan was responsible for extract preparation, animal experimentation, and data collection, Elfred Rinaldo Kasimo performed laboratory analyses, including cytokine measurement and data validation, Rochmad Krissanjaya contributed to statistical analysis, result interpretation, and critical revision of the manuscript, and all authors have read and approved the final manuscript.

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